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STUDIES ON LETTUCE GROWTH IN
CONTROLLED ENVIRONMENT FACILITIES

by



SENG PAI (ALLEN) LANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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DEPARTMENT OF PLANT SCIENCE

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THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certified that they have read,
and recommend to the Faculty of Graduate Studies and
Research, for acceptance, a thesis entitled.....
Studies on lettuce growth in controlled
.....
environment facilities.
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submitted by SENG PAI (ALLEN) LANG
.....
in partial fulfilment of the requirements for the degree
of Master of Science.

ABSTRACT

The object of this project was to find the optimum balance of environmental factors such as light intensity, photoperiod, nutrient level, CO₂ concentration and temperature in order to get maximum yield of butterhead lettuce.

The system for monitoring environmental factors was made as automatic as possible. The growth chambers contained built-in controls for temperature, relative humidity, light intensity and photoperiod. Two other systems were introduced for controlling the carbon dioxide concentration and for supplying the nutrient solution to the pots. The CO₂ control system included an IRGA, an electronic controller, a recorder, valves and tubing, thus permitting the CO₂ concentration in the chamber to be maintained at 1500 ppm. The automatic watering system included two timers, two carboys, tubing, a valve, solenoid valve and a filter. The two timers were adjusted for twice daily watering to give every pot in the chamber 700 ml nutrient solution per day.

Lettuce (Lactuca sativa L.) cv. Buttercrunch growing under conditions of 1500 ppm CO₂, 20C constant temperature, 2000 fc light intensity and a 16 hour photoperiod was successfully matured in 27

days from seeding. The cultivar, *Ostinata* was successfully grown under the same conditions used for cv. *Buttercrunch* but a photoperiod of 18 hours would probably be advantageous.

Butterhead lettuce, when grown in greenhouses, takes at least 60 days from seed to maturity. Therefore, maturation in 27 days represents a considerable saving of time and hence the possibility for greater yields per unit area per year. Lettuce grown under 16 and 24(2 wks)/16(2 wks) photoperiod regimes had more fresh weight and less dry matter content than those grown under a photoperiod regime of 24 (continuous light) or 16(2 wks)/24(2 wks) hours.

The accumulation of free NH_4^+ in the tissue was positively correlated with lettuce bitterness. The data also suggested that the reduction of chlorophyll in lettuce growing under continuous light was light intensity dependent. The leaf length/width ratio was greater than one at lower light energy levels and less than one at higher light levels.

A major problem that arose in the course of attempting to speed up the growth rate of the lettuce was tipburn. Tipburn is a breakdown of tissue at the margins of newly forming leaves in the later stages of maturation or heading. In these experiments, the severity of tipburn was closely correlated to growth

rate.

A possible cause of tipburn is rupture of cells near laticifers. At high light intensity levels, the assimilation activity in lettuce leaves is very high, resulting in increased assimilates in the laticifers of young leaves. This increase in assimilation is coupled with rapid growth resulting in increased demands for calcium and/or boron. This in turn can lead to a shortage of soluble Ca resulting in poor cohesiveness between newly forming cells. The assimilates accumulate within the laticifers increasing solute concentration and thereby increasing osmosis and turgor pressure resulting in laticifer bulging (Tibbitts and Read 1976). Because of a lack of available Ca, plants cells are weak, union of laticifer and parenchyma cells takes places and parenchyma cells fill with latex resulting in leaf necrosis.

Using a modified solution eliminating ammonium ions (Appendix II) as well as reducing both light intensity and photoperiod was found to effectively eliminate the bitterness problem.

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INTRODUCTION

With increasing food prices and much of this due to transportation costs, it is important that we attempt to grow more of our food close to the market. Furthermore, with the present development of more efficient means of converting electric energy into light energy, the time is approaching when the production of fresh vegetables in controlled environment facilities may be economically feasible.

The availability of light, either in terms of light intensity, photoperiod or light quality, is often the most significant determinant of plant productivity. Since plant production depends upon the continuous supply of various factors such as light, carbon dioxide, temperature and mineral nutrients, Blackman's (1905) principle of limiting factors should operate i.e. "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor". Accordingly, the interaction of light and other environmental factors should produce a curve showing exponential and saturation growth (Blackman 1905). The object of this work was to try to find the saturation light intensity under conditions of 1500 ppm carbon dioxide, twice daily application of nutrient solution and a constant temperature of 20C. The levels of the parameters other than

light were chosen on the basis of previous work which indicated that these levels were probably near optimum for maximum growth and yield of lettuce.

Some disorders of lettuce growing under controlled environment conditions have been encountered by other workers (Kurelek unpublished thesis). Such disorders have included tipburn and bolting in particular. A further aim of this work was to determine how to eliminate these disorders and still maximize growth and yield.

LITERATURE REVIEW

I. Growth Rate

Phenomenal plant yield, 10 to 50 times greater than normal yield (dry weight) has been obtained with improved cultural practices and the growing of plants under controlled environment conditions(Krizek, Klueter and Bailey 1969). Although many researchers and growers have stimulated plant growth 2 to 3 fold by varying individual environmental parameters such as light intensity, photoperiod or nutrient levels, a research team at Beltsville, Md., was the first to control the combination of all of these factors in a specially-designed growth chamber(Krizek, et al 1969). In addition, they developed a new precise schedule for seedling production:

1. seeds are planted directly to pots. Seedlings are never transplanted, only thinned.
2. environmental treatments are started very early, at the time of seeding or when the seedlings are no more than 4 to 10 days old.

Using higher temperature 85/75F(29/24C), light intensity at 4000fc(43.2 klx with fluorescent lamps supplemented by incandescent lamps), relative humidity of 65% and carbon dioxide concentration at 2000 ppm, air speed of 40 ft/min.(12.2m/min.) and nutrient solution applied four times per day, this research team was able to obtain petunia yields(dry weight) 1,000 to 5,000% greater than

yields from plants conventionally grown (Krizek, et al 1969).

Burpee Hybrid cucumber, Michigan - Ohio tomato and Grand Rapids lettuce were grown by Krizek, Bailey, Klueter and Liu for 15 days at elevated temperatures (30/24C), light intensity of 43.2 klx (4000fc) for a 16 hour photoperiod at carbon dioxide 2000 ppm and 65% relative humidity. Plants were fertilized hydroponically four times per day. At the end of the experiment, lettuce plants were two times heavier (dry weight) than those grown under standard environmental conditions in the growth chamber (24/18C, 31.5 klx of light and 400 ppm carbon dioxide) and 10 times heavier (dry weight) than those grown in the greenhouse under natural day light (Krizek, et al 1972). Generally, an increased seedling growth was obtained when temperature, light and carbon dioxide were increased simultaneously.

Work at Naaldwijk (as summarized by Canham 1966) showed that a 50% increase in yield (fresh weight) of lettuce was obtained with four hours supplementary light per day during winter greenhouse production. Dennis and Dullforce (1972) grew lettuce in growth chambers during a short propagation period. This lettuce was 16 times greater in dry weight after 12 days in the growth chamber than greenhouse propagated seedlings.

Soffe, Lenton and Milton (1977) grew seven vegetable species (lettuce, beetroot, spinach beet, cabbage, winter oilseed rape, radish, and celery) in controlled environments using three treatments namely (A)- 12 hours per day of visible irradiance(115 Wm^{-2}) from fluorescent and incandescent lamps, (B)- 16 hours the same type of light at 88 Wm^{-2} and (C)- 16 hours of treatment A with 4 hours of low intensity incandescent light(3 Wm^{-2}). Dry weight in all seven species was increased by extending the day length from 12 to 16 hours(A to B). The dry weight of lettuce heads was doubled and their size increased. Furthermore, the dry weight of lettuce heads was doubled in treatment C compared with treatment A.

II. Light

Dullforce (1971) grew butterhead lettuce cultivar Cheshunt 5B under light intensities ranging from 65 to 650 lumen ft^{-2} or foot candle(approximately 3.5 to 35 $\text{cal cm}^{-2} \text{ day}^{-1}$ visible radiation). These were grown under 16 hour photoperiod using day light and warm white lamps. The temperature treatments were kept at 13, 16, or 21C. At 21C, heading occurred only at the highest light intensity; at 16C, heading would occur with light as low as 12 $\text{cal cm}^{-2} \text{ day}^{-1}$ and at 13C, as low as 8.2 $\text{cal cm}^{-2} \text{ day}^{-1}$.

The relative growth rate of lettuce can be very fast under well-balanced light and temperature (e.g. $35 \text{ cal cm}^{-2} \text{ day}^{-1}$ at 21°C). A relative growth rate value of over 30% per day was recorded in the early stages (Dullforce 1971).

Heath and Meidner's results(1967) with cultivar Grand Rapids indicated that the lettuce plants was able to gain dry weight in very low light conditions. According to Monteith's statement(1965), the efficiency of energy conversion was 8 to 14% of total light energy available to the plant in weak light intensities. A conversion rate of 11% was found at the lowest light intensity used in Dullforce's experiment($3.5 \text{ cal cm}^{-2} \text{ day}^{-1}$)(Dullforce 1971).

Some work has been done on the combination of light intensity and photoperiod as it affects lettuce seedlings. Long days(16 hours) at a low light intensity can result in larger leaf area than 8 hours at approximately double the illumination (Verkerk and Th. Spitters 1973).

Bensink(1971) did some very thorough studies in the morphogenic development of lettuce leaves in response to light and temperature. The number of leaves produced could be correlated with light intensity up to $100 \times 10^3 \text{ ergs/cm}^2 \text{ sec}$. AT a light intensity of about $1000,000 \text{ ergs/cm}^2 \text{ sec}$, an average of 2.5 leaves is

initiated per day. At $7000 \text{ ergs/cm}^2 \text{ sec.}$ only 0.4 leaves per day are formed. Leaf elongation was stimulated mainly as a result of low light intensity- this phenomenon helps to explain the difficulty of hearting in winter grown butterhead lettuce. Leaf width was positively affected by light energy, either high light intensity or longer day length. Midrib elongation was suppressed by high light intensity (Bensink 1971).

III Temperature

Dullforce (1971) used the growth analysis method to calculate the efficiency of energy utilization. There was some evidence that energy conversion was more efficient at 13C than 16C or 20C. Verkek and Th. Spitter (1973) found that lettuce (leaf area) responds to temperature in the following order: $25/17 > 21/21$, $21/13 > 17/17$, $17/9 > 13/13$. High temperature resulted in larger leaf area and more leaves produced.

Bensink (1971) using the butterhead Bibb type lettuce cv. Meikoningen at three different light intensities found that leaf production is temperature and light intensity dependent. Leaf production at all light intensities was higher at high temperature and it is likely that at a low energy level of the plant, i.e. at low light intensity, reduction in width may result from increased competition for growth substances.

Rajan and Blackman (1975) compared the interacting effects of light and temperature on growth in the vegetative phase of Gossypium hirsutum, Helianthus annuus, Phaseolus vulgaris and Zea mays. Temperature was either maintained constant at 20 and 30C or reduced at night by 5 and 10C and the plants subjected daily to either 2.16 or 4.32×10^4 klx of light for 14 hours. Relative growth rate (RGR) was not favoured by cool nights. Such reduction in temperature retards the growth process to a varying degree according to the species.

IV. Carbon Dioxide Enrichment

Dullforce (1965) conducted experiments using a three-fold increase in the carbon dioxide concentration and noted its effects on the growth and development of lettuce. She observed that the difference in growth in the early stages was much more marked than in later stages. It was suggested that the effect of supplemental carbon dioxide was less striking when the lettuce plants became covered with leaves, because at this stage of development, the rate of diffusion of carbon dioxide enriched air to the plants would be reduced and many stomata would be closed due to the shade induced by overlapping leaves.

Gardner, as reported by Skoye (1972), found that the combination of warm day temperature and carbon dioxide enrichment was responsible for early maturity of lettuce and the formation of better-hearted plants.

However, high day temperature in the absence of supplementary carbon dioxide delayed the maturity of lettuce from four to seven days and produced lettuce of inferior quality.

Wittwer and Robb (1964) conducted carbon dioxide enrichment investigations with tomato, lettuce and cucumber. In all plantings, carbon dioxide enrichment resulted in increased weight. The maximum response of lettuce to carbon dioxide enrichment was a 70% increase in fresh weight and a 120% increase in dry weight.

V. Relative Humidity

Tibbitts and Bottenberg (1971) conducted temperature-tipburn relationship studies in which the conditions used were 70F (21C), 70% R.H. and 85F (29C), 50% R.H. It was found that when the vapor pressure deficit was increased from 7 to 24 mb, it resulted in increased tipburn but not so rapidly as with temperature increase alone. Tibbitts and Bottenberg (1976) grew lettuce in two relative humidity regimes (85% and 50%) and found a faster growth rate under 85% than 50%. The higher humidity level increased leaf number 15%, leaf size 30%, dry weight 62%.

VI. Problems of Lettuce Grown in Controlled Environment Conditions.

A. Tipburn

In the past, lettuce was grown predominantly as a spring crop, the term tipburn was used to describe a physiological condition frequently found on outdoor grown spring lettuce. The symptoms of normal tipburn are visible on the leaves enclosing the head and on the leaves just underneath. The first symptoms are small transparent lesions along the small veins near the leaf margins. The leaf margins become flaccid and afterwards turn brown (Termohlen and van den Hoeven 1965).

Environmental factors are known to influence the development of lettuce tipburn markedly. One consistent response from all the previous studies on lettuce is that rapidly growing plants are most susceptible. When temperature, moisture, light and nutrition are near optimum for the growth of the plants, tipburn is severe (Tibbitts and Rao 1968). Plants growing under illumination of 1800 fc for 20 and 24 hours daily develop a more severe tipburn than plants growing under a reduced light intensity of 800fc and under shorter light periods of 16 and 8 hours. Injury is closely associated with the rate of plant growth. The effect of light in inducing tipburn appears to be an indirect effect of increased plant growth rate and dry matter accumulation (Tibbitts and Rao 1968). Tibbitts and Bottenberg(1971)

studied the effect of temperature on tipburn and found that higher temperature caused increased tipburn of Bibb type lettuce. Cox, McKee and Dearman(1976) found that the occurrence of tipburn can be closely related to temperature when light intensity is constant. Symptoms can be delayed if light intensity is decreased at a given temperature.

A possible reason why tipburn is related to relative growth rate (RGR) is because with a high assimilation activity in the leaves associated with rapid growth rate, the demand for nutrients by the plant is increased. This can lead to a deficiency of certain elements, particularly in younger leaves. Since the mobility of calcium is low, it seems likely that calcium deficiency may be involved rather than other factors which could be correlated with RGR such as leaf vascularization, cuticle development and stomata spacing (Cox, et al 1976). However, the uptake and utilization of calcium in the plant depends not only on the presence of calcium ion in the soil solution of the rhizosphere but also on the presence of other accompanying ions and the general constitution of the plants (Cox, et al 1976; Sonneveld and van den Ende 1975). Calcium is an essential component of pectinacious plant substances. A deficiency will result in poor cohesiveness between cells, especially

in rapidly growing tissue. Calcium movement studies have shown that Ca has to be supplied continuously for adequate distribution to newly forming tissue. Calcium is also a membrane stabilizer and affects cell permeability. The availability of relatively immobile Ca is critical for developing tissue. Symptoms of calcium deficiency may occur even with an ample supply of calcium in the soil (Ashkar and Ries 1971; Carolus 1975).

Mineral analysis has indicated that Na, Mg and NH_4^+ in the nutrient solution may compete with Ca reducing Ca content of the plant tissue and in turn resulting in tipburn necrosis (Sonneveld and van den Ende 1975). Kruger (1966) grew lettuce cultivar Pennlake in sand culture using a Hoagland's nutrient solution. Calcium concentrations of 34, 194 and 268 ppm were used. Higher Ca levels resulted in increased dry weight. Foliage spraying with Ca significantly reduced tipburn occurrence, and through tissue analysis it was found that considerable Ca was taken in by the leaves. Thibodeau and Minotti (1969) using tipburn susceptible Bibb type lettuce cultivar Meikoningen applied Hoagland No. 1 nutrient solution daily in a sand culture experiment. They sprayed 0.04 M $\text{Ca}(\text{NO}_3)_2$ on leaves and found that it completely controlled tipburn. Ashkar and Ries (1971) working with the cv. Great Lakes

under both greenhouse and growth chamber conditions found that nitrate ion and amino acid level were both higher in tipburn tissue than normal tissue. Furthermore, no tipburn occurred when Ca was present in the growing medium.

Crisp, Collier and Thomas (1976) found that boron deficiency induced tipburn. They reported that auxin activities were independent of boron levels for much of the time, but auxin-1 activity was increased by boron deficiency shortly before the onset of tipburn. There was no evidence that boron content affected calcium content in the leaves.

Increased boron in the nutrient medium, particularly at low Ca levels, increased growth. Boron apparently increased the permeability of the plant's roots to Ca. Lettuce plants that were susceptible to tipburn contained a higher ratio of insoluble Ca (Ca oxalate) to soluble Ca in rapidly growing lettuce leaves than in outer leaves (Carolus 1975). Sonneveld and van den Ende (1975) found that sodium bicarbonate in the nutrient solution will cause a greater yield reduction in lettuce and a greater severity of tipburn than other salts (e.g. NaCl, NaNO₃, Na₂SO₄, CaCl₂, MgCl₂, KCl). Potassium chloride did not increase tipburn and calcium chloride almost completely prevented this disorder.

In lettuce leaves, there are meristematic cells located along the two margins of the leaf axis (midrib) called marginal meristems. The activity of these meristems starts about $1/3$ down from the tip of the leaf (midrib) and gradually extends along the whole axis (Bensink 1971).

Rupture of laticifers in the leaves of lettuce has been reported to be responsible for tipburn (Olson, Tibbitts and Struckmeyer 1967). The laticifers of lettuce are the articulated type. At maturity, they are branched, open vessels (Hayward 1938). The phenomenon of laticifer rupture is commonly preceded by abnormal bulging brought on by the accumulation of the assimilates within the laticifers under high light level followed by increased water uptake (Tibbitts and Read 1976). Union of laticifers with adjacent parenchyma cells then takes place. The laticifer-parenchyma cell unions commonly occur at localized bulges and result in the filling of parenchyma cells with latex. These cells frequently rupture and release latex into the surrounding tissues (Tibbitts and Read 1976).

This phenomenon of bulging and rupturing has been observed only in the young developing leaves during laticifer differentiation. These leaves are devoid of chlorophyll, therefore carbohydrate

accumulation would have to be a result of transport from green leaves (Tibbitts and Read 1976).

B. Chlorosis

A prolonged daily exposure to light can prevent chlorophyll formation in the leaf, thereby, revealing more of the yellow carotenoid pigments and causing chlorosis. In tomato, chlorophyll development is inhibited when plants receive more than eighteen hours of daylight per day. Under continuous light no chlorophyll is formed and the leaf is completely blanched (Hillman 1956).

Too high a light intensity impairs photosynthesis by rapidly photo-oxidizing chlorophyll, so that the remaining available supply is inadequate to absorb sufficient light energy. As the old chlorophyll is destroyed in the most exposed upper palisade cells, leaf color fades. High light intensity (9,300-12,000 fc) may also inhibit chlorophyll synthesis, further limiting the chlorophyll content (Treshow 1970). Continued exposure to intense light may kill the protoplasts of the leaf, stem or fruit cells and cause browning of localized areas of tissue (Treshow 1970).

Tomato plants subjected to 24 hours of irradiance at light intensities as low as 4 fc became chlorotic (Hillman 1956). This result could be interpreted in terms of Bünning's (1952) theory of

endogenous daily rhythms, since it appears that cycles differing too greatly from a 24-hr periodicity are injurious (Hillman 1956).

Daily temperature changes (e.g. 16 hr at 30C., 8 hr at 17C) can prevent injury by continuous light, the temperature effects fit into this concept. In general, the normal leaf development of tomato requires some environmental fluctuation with a period close to 24 hr. supplied either by light-dark relations or sufficiently wide temperature changes (Hillman 1956).

C. Bitterness

In field production of lettuce a bitter principle often develops in the leaves, particularly under high temperature conditions (Whitaker, et al 1974). No references could be found dealing with the chemical nature of the bitter principle in lettuce. However, it is assumed that the bitterness is probably due to the presence of an alkaloid (Robinson 1968) or possibly a bitter peptide (Arai, Yamashita, Kato and Fujimaka 1970).

In general, alkaloids tend to accumulate in 1) very biochemically active tissue, 2) epidermal and hypodermal tissue, 3) vascular sheath and 4) latex vessels. They tend to accumulate in cell vacuoles rather than the surrounding cytoplasm.

Environmental factors have been found to

influence alkaloid content. Light energy is a major factor in increasing total alkaloid content, but for the most part its influence is indirect. Nitrogen supplied to plants in the form of ammonium salts rather than nitrates increases alkaloid yield (Robinson 1968) .

VII. Plant Response Growing Under Continuous Light

Bate and Canvin (1971) compared the effect of photoperiod on the growth of young aspen trees and found that, provided the photoperiod was longer than that required to maintain active growth, further increases in the length of photoperiod would result in increasing the rate of dry matter accumulation. His data also suggested that aspen trees should have the greatest dry matter gain if grown under continuous illumination from fluorescent lamps at 15C.

VIII. Effect of Light on Root Growth.

Leopold and Kriedemann (1975) have reported studies at the Auburn Rhizotron (root-observation chamber at Auburn University, Alabama) providing a vivid demonstration of diurnal changes in root extension and contraction in a time lapse movie. Plant root extension ceases during the day light hours, and because of low turgor, roots contract and break contact with the soil particles but after sunset,

turgor is restored and extension growth re-established.

MATERIAL AND METHODS

I. Growth Chamber Description

The growth chamber used for these experiments was a step-in type, Model M-12, designed by Environmental Growth Chambers of Chargin Falls, Ohio. The light sources for all the experiments were a combination of fluorescent lamps and incandescent lamps. The fluorescent lamps were cool white type, 1500 mA, eight feet long. The incandescent lamps were 60 watt, 120 V. Between the growing area and the lamps was a plexiglass barrier. This barrier was loose fitting around the edges. For the experiments, the barrier was taped to the sides of the chamber with Scotch tape to effectively seal the growing area from the lamp housing. The reflective wall surfaces were made of specular aluminum. The pots used in the experiments were covered with a layer of aluminum foil for better reflection of light. The position of the bench (elevation) was adjustable.

II. Control of Light

Photoperiod was controlled by a time clock. Lights were turned on and turned off abruptly.

Light intensity was measured in foot-candles (fc) with a Weston illumination meter (model 756. Quartz filter) without cosine correction. Fig. 1 shows the light intensity distribution inside the chamber at a

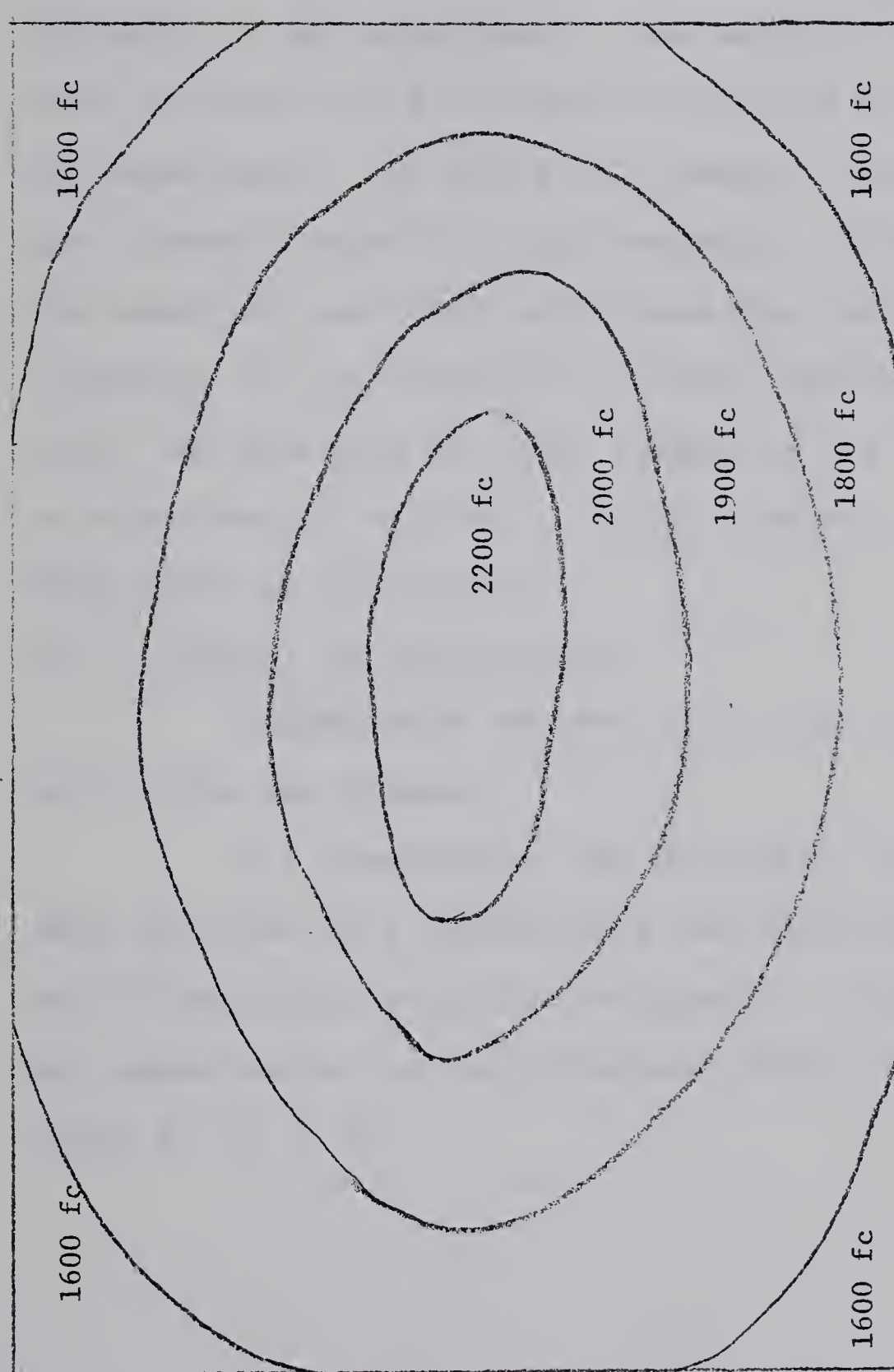


Figure 1. Diagram showing the distribution of light intensity within the growth chamber at bench level (90 cm).

distance of 90 cm between barrier and bench.

it becomes obvious that due to the decay of lamps and the growth of plants, light intensity will be variable in any experiment. One method of reducing this variability is to adjust the bench height during the experiment. By doing so, however, other factors are changed, especially air movement. So we maintained the bench at one level which gave the desired light intensity at the beginning of each experiment. Therefore, the changing of light intensity was included as an experimental variable. Light intensity readings were taken at pot height.

III. Control of Temperature

Temperature control units were automated and built into the chamber.

Air temperature and dew point temperature were recorded on a temperature recording chart, (Honeywell temperature recording equipment). Temperature in all experiments was kept constant during the day and night at $20 \pm 2^{\circ}\text{C}$.

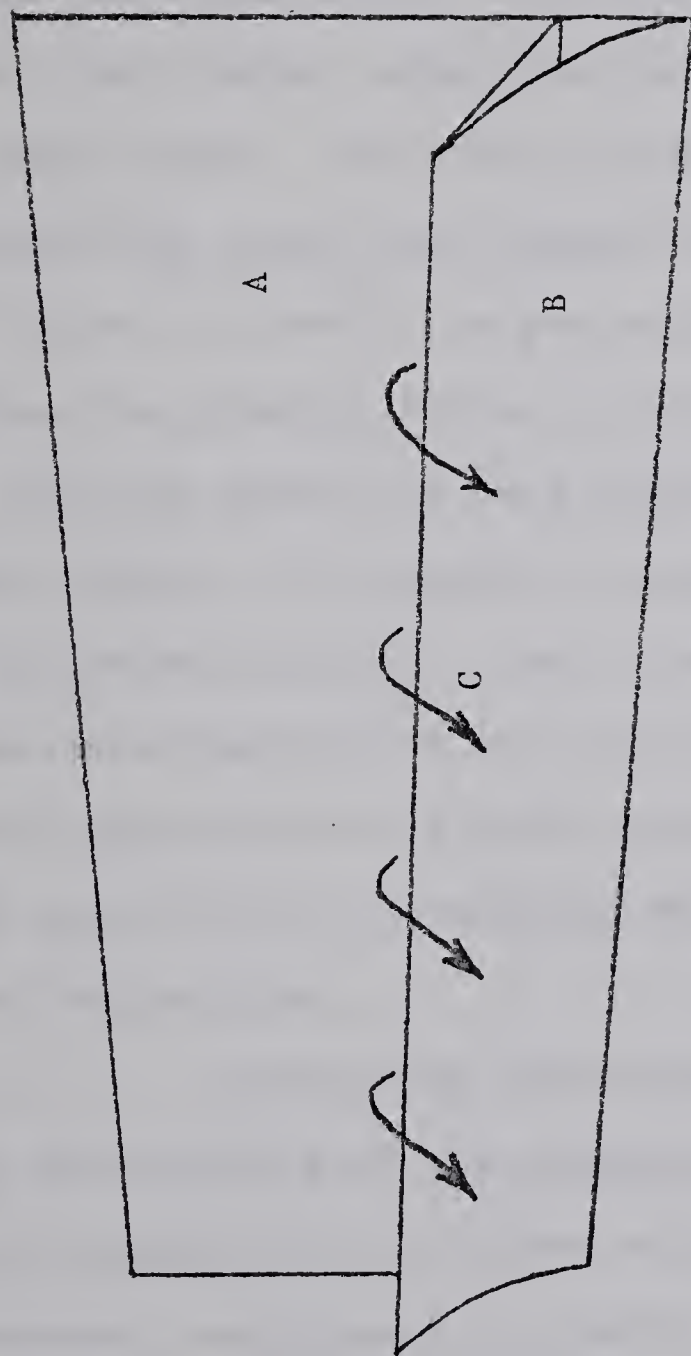
IV. Control of Air Movement

The speed and pattern of air movement varied over the growing area and depended upon the position of the bench. The higher the bench, the higher the air speed and the greater the leaf flutter of the plant material. To avoid direct air blow on the seedlings, a baffle was constructed. The baffle consisted of a layer of cardboard covered with aluminum foil (see Fig. 2). After this adjustment, there was slight fluttering only at the very early stages of plant growth. None was visible after two weeks. Air flow speed in the chamber was approximately 30.5m/min at plant level with a Hastings air meter. The direction of flow of air was downward.

V. Control of Relative Humidity

Relative humidity was controlled by adjusting air and dew point temperatures. However, because of the poor reliability of the control system, a psychrometer was used in order to ensure that relative humidity was correctly adjusted.

Relative humidity was kept at 85% day and night. Throughout the experiments performance was reasonably accurate and consistent. Sometimes, however, there were intervals when relative humidity was too high, due to mechanical problems connected with steam input or leakage from the freezing unit. Since the relative



A: end wall grill housing for fans

B: cardboard baffle lined with aluminum foil

C: direction of air flow

Figure 2. Diagram showing the mounting of the cardboard baffle on the end wall of the growth chamber to deflect the flow of the air from the circulating fans.

humidity was always 85% or higher, this problem likely had very little if any detrimental effect on plant performance. For all the experiments, condensation of moisture on the plants was never observed.

VI. Growing Containers and Growing Medium

Thirty 20 cm diameter plastic pots were used for the experiments. The pots were spaced evenly throughout the chamber along the two sides of a central irrigation header tube. (See VIII, watering equipment.) Pots were carefully soaked and washed between each experiment. A layer of gravel was put on the bottom of each pot, then the growing medium to within 2.5 cm of the top edge. A certain amount of hand pressure was required to compress the medium. Two types of growing medium were used: peat-vermiculite (1:1 by volume) and pure peat. The peat was pre-soaked with hot water before being used. Dolomite was incorporated to bring the pH of the media to 6.3. No sterilization treatment was given to either the peat or vermiculite.

During the experiments, some algae developed on the surface of the growing media, probably a result of contamination from the air or from the handling. However, no disease or insect problems were encountered.

VII. Seeding Technique

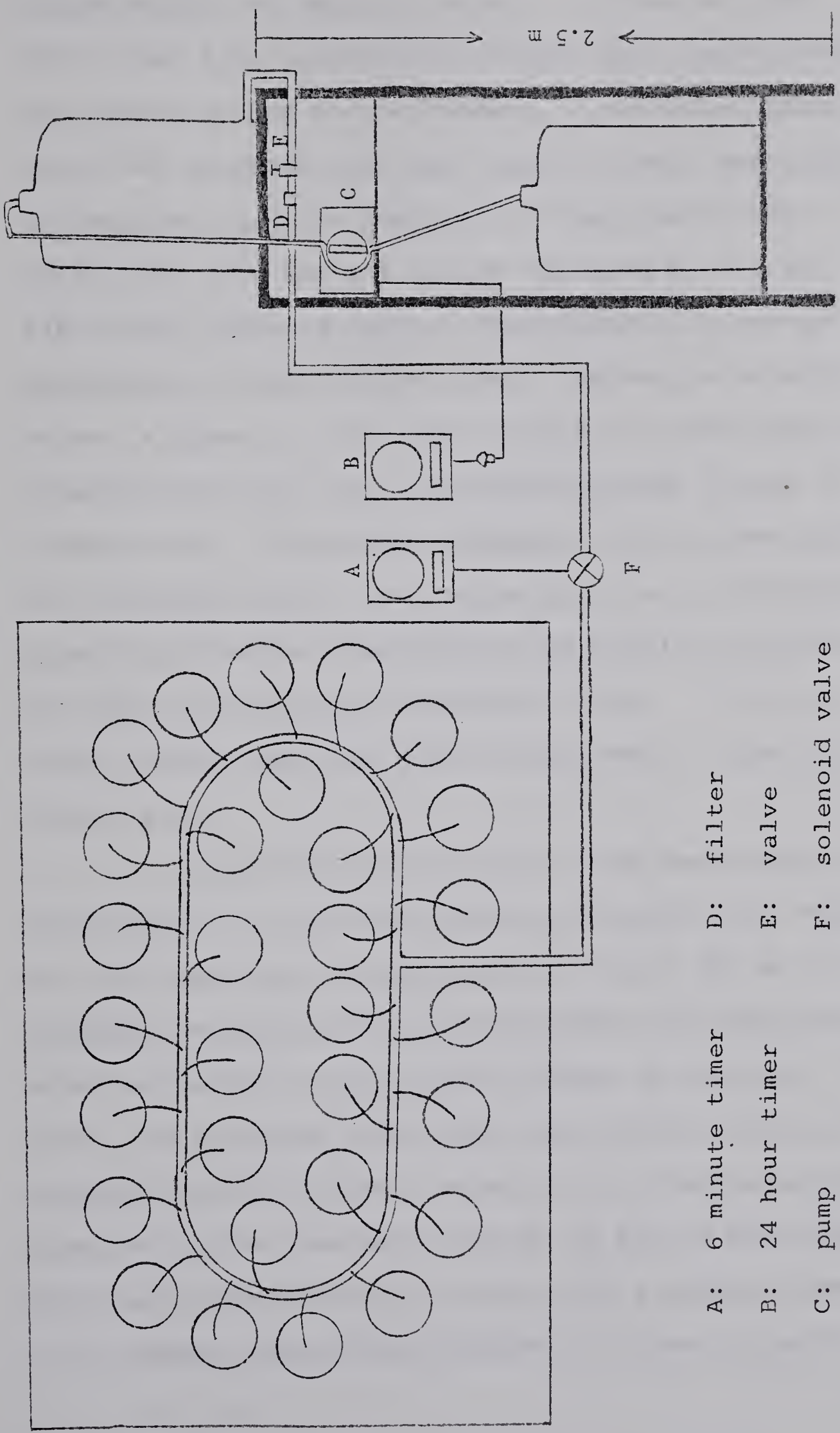
In these experiments, three cultivars of butter-head lettuce were used: Buttercrunch, Ostinata and

Deci-minor, but not all cultivars were used throughout. Before seeding, the pots of growing media were soaked in water overnight. Three seeds were planted at the center of each pot, approximately 2.5 cm apart in a triangular pattern. At the end of the first week, one seedling was removed from each pot and the remaining two seedlings allowed to develop. After two weeks, another one was removed to allow only one to grow to maturity.

VIII. Watering Equipment

The application of water and nutrient solution to the experimental plants was accomplished by use of an automated watering system. This system consisted of two timers (the first on a 24 hour basis, the second on a 6 minute basis), a 3/4" (18.75 mm) I.D. (Internal Diameter) solenoid valve, a manually operated valve, a filter, two 50 liter carboys and a varistaltic pump (pumped the nutrient solution from lower carboy to supply carboy). This equipment was installed outside the growth chamber making it unnecessary to disturb the plant's environment during application of the nutrient solution. See Fig. 3.

Final delivery of the nutrient solution was by gravity through a Chapin "heavy weight supply header" containing .076" (1.9 mm) I.D. "spaghetti" tubes. The lead weight on the end of each supply tube was placed



- A: 6 minute timer
- B: 24 hour timer
- C: pump
- D: filter
- E: valve
- F: solenoid valve

Figure 3: Diagram of the nutrient and irrigation system showing the distribution of pots on the growth chamber bench.

in the middle of each 20 cm pot. A piece of 3/4" (18.75 mm) I.D. polyethylene tubing was used to connect the supply carboy to the header. A manually operated valve was inserted into the tube to permit servicing of the line, e.g. to replace or clean the filter. Below the valve was the filter followed by a 3/4" (18.75 mm) solenoid valve. The solenoid valve was connected to the 6 minute timer. Below the solenoid valve, a piece of 3/4" (18.75 mm) I.D. rubber pipe was inserted into the line and passed through a hole in the chamber wall. Inside the chamber, the line was connected to the header pipe. The header pipe was a continuous ring to give equal distribution of nutrient solution through the capillary "spaghetti" tubes. Thirty of these Chapin capillary tubes were used to water the thirty pots.

From the observations at the beginning of the experiments, it was found that 4.5 minutes was required at each watering to supply each pot with 350 ml of nutrient solution. The minimum activation time possible with the design of the 24 hour timer is one hour. Therefore, the 6 minute timer had to be calibrated to run intermittently to give a total of 4.5 minutes activation time during the one hour setting of the 24 hour timer. This was accomplished by setting the 6 minute timer on a 27 seconds cycle that repeated 10 times during the

1 hour activation period.

Since two daily waterings were desired, the 24 hour timer was set to activate at 4 a.m. and 4 p.m.

In this technique, no additional pressure was necessary to deliver the nutrient solution to each of the pots. The pressure due to gravity was high enough to deliver the desired amount of nutrient solution using the previously described timer control system.

Delivery of equal volumes of water or solution to each pot was the crucial test for this system. Practice runs were made before every experiment and it was found that the uniformity of distribution was good. Differences in delivery between pots were within 5%.

IX. Drainage System

Besides the dearth of published information on watering systems for growth chamber experiments, virtually no information could be found on management of excess water under controlled environment conditions. It is obvious that excess water inside a growth chamber will influence the humidity control system and increase the risk of damage to the growth chamber equipment, especially the air pumping system, by encouraging the growth of fungi and algae and also salt corrosion (Kurelec unpublished thesis). Since the growth chamber used here did not provide any drainage system to drain excess water from the floor, a closed drainage system for all the pots had to be provided.

Wide mouthed shallow bulbpanns provided close fitting and stable support for the pots used. Three out of the four holes in the pan were sealed and the fourth hole was fitted with a coupler for tygon tubing. A layer of nylon screen was glued along the inner edge of the bulbpan insuring that no debris or medium could pass through to block the drainage system.

A length of tygon tubing was fitted to each coupler (projecting below the bench) and all of the tubing was directed outside the chamber. A carboy was used outside the chamber to collect the used nutrient solution. Another piece of rubber tubing was used to direct the used solution to a nearby drainage sink.

X. Carbon Dioxide Regulation System

The apparatus and equipment for maintaining the carbon dioxide inside the chamber at a fixed level (i.e. 1500 ppm) included:

One Infrared Gas analyzer. A Beckman 865 model. (IRGA)

One recorder, Beckman 10" recorder.

One solenoid valve to open and close the flow of CO₂ from the CO₂ tank to the growth chamber.

Five needle valves to control the volume of CO₂ flow.

One electronic controller, one air pump, one flow meter.

One variastat, two plastic tubes containing desiccant and tygon tubing (see Fig. 4).

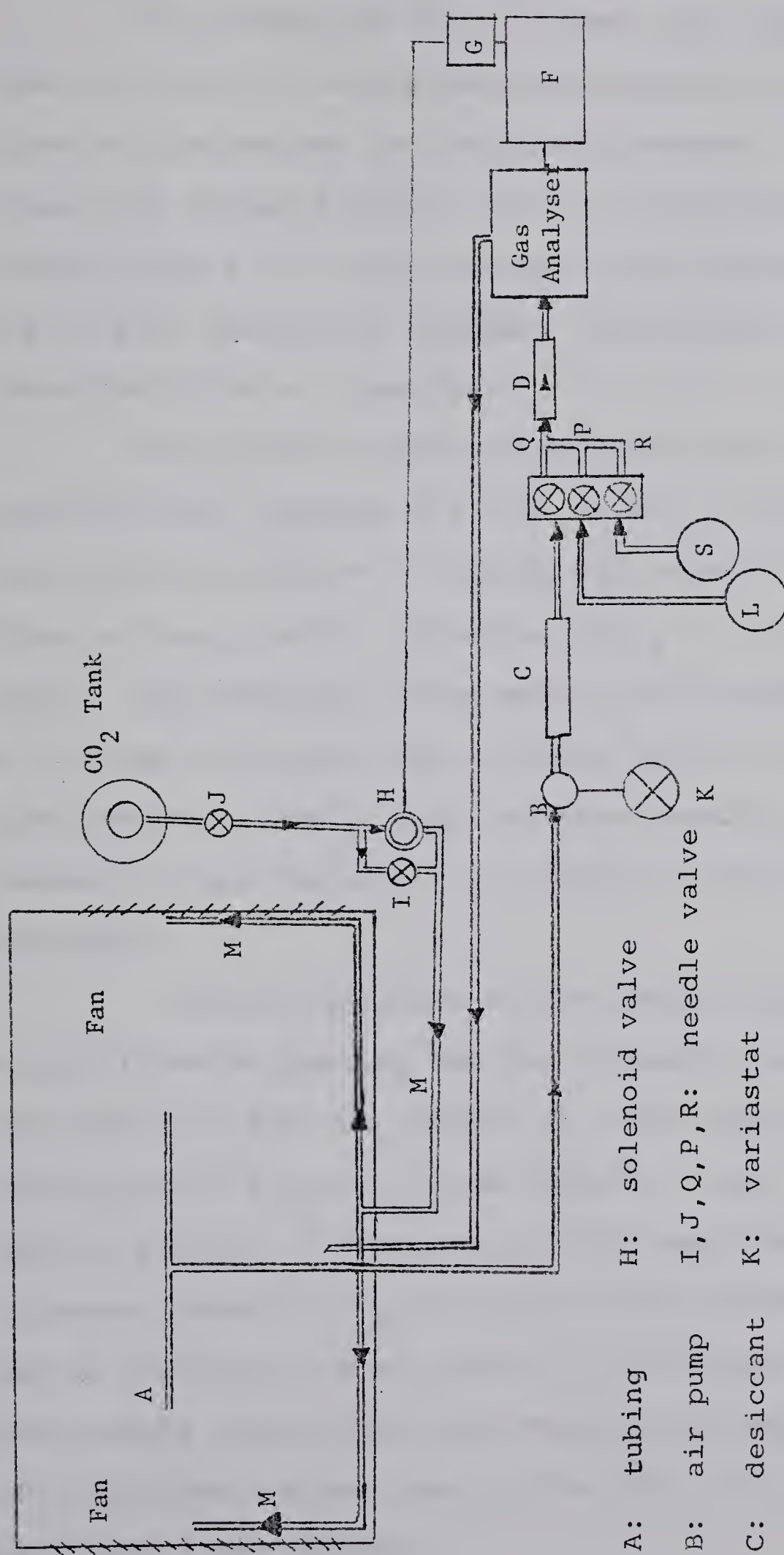


Figure 4: Diagram of the CO₂ control and the distribution system.

A. Carbon dioxide sampling system.

Two pieces of 5/16" (7.8mm) I.D. tygon tubing (see A in Fig. 4) were placed horizontally along the two sides of the benches in the growth chamber. These two tubes were joined together with a third piece of tygon tubing using a "Y" shape connector and passed through the wall of the growth chamber. This tubing was connected to an air pump (B).

The speed of the air pump was controlled by a variastat (k), keeping the flow of CO₂ to the infrared gas analyzer constant. The CO₂ was passed through two tubes of desiccant (C) (granular CaSO₄) to remove water vapor. The desiccant tubes were 2 cm in diameter and 20 cm long with glass wool in each end to remove dust from the air. Due to high relative humidity inside the chamber, it was necessary to change the desiccant every two days.

Final regulation of the sample flow rate was accomplished by passing the gas through a needle valve (Q) and a S.C.F.H. CO₂ meter (D). The flow rate was controlled at 8 p.s.i. (0.56 kg/cm²). Some trials were made to find the difference in IRGA readings due to different rates of CO₂ flow and it was found that there was no difference up to 20 p.s.i. (1.41 kg/cm²). Following passage through the flow meter, the sample CO₂ passed into the IRGA. After passing the IRGA, the gas was returned to the chamber.

A Beckman 10" recorder (F) was connected to the IRGA. It recorded the CO₂ concentration from the IRGA at a constant speed (e.g. .1 in/min. 2.5 mm/min.) A controller (G) was devised to coordinate the reading of the IRGA with the rate of flow of CO₂ into the chamber and thereby keep the concentration of CO₂ in the chamber constant. The main components of the controller were: a relay, Infrared sensor, transistors and resistors. The circuit diagram of the controller is shown in Fig. 5.

The mode of action of the controller is as follows:

The infrared sensor was able to detect the difference between two distinct background colors, e.g. black and white. The infrared sensor was connected to the relay (see Fig. 6). The power of the relay was supplied by a 12 D.C. power supplier.

The opening and closing of the relay was controlled by the infrared sensor. Normally, when an infrared sensor reads a black area, it doesn't reduce the interval resistance and no current will flow through. If, however, the infrared sensor reads a white surface, internal resistance is reduced and enough current flows through to close the relay. The controller is connected to solenoid valve (H). When the relay is closed, current will flow through resulting in the opening of the solenoid valve (Fig. 6).

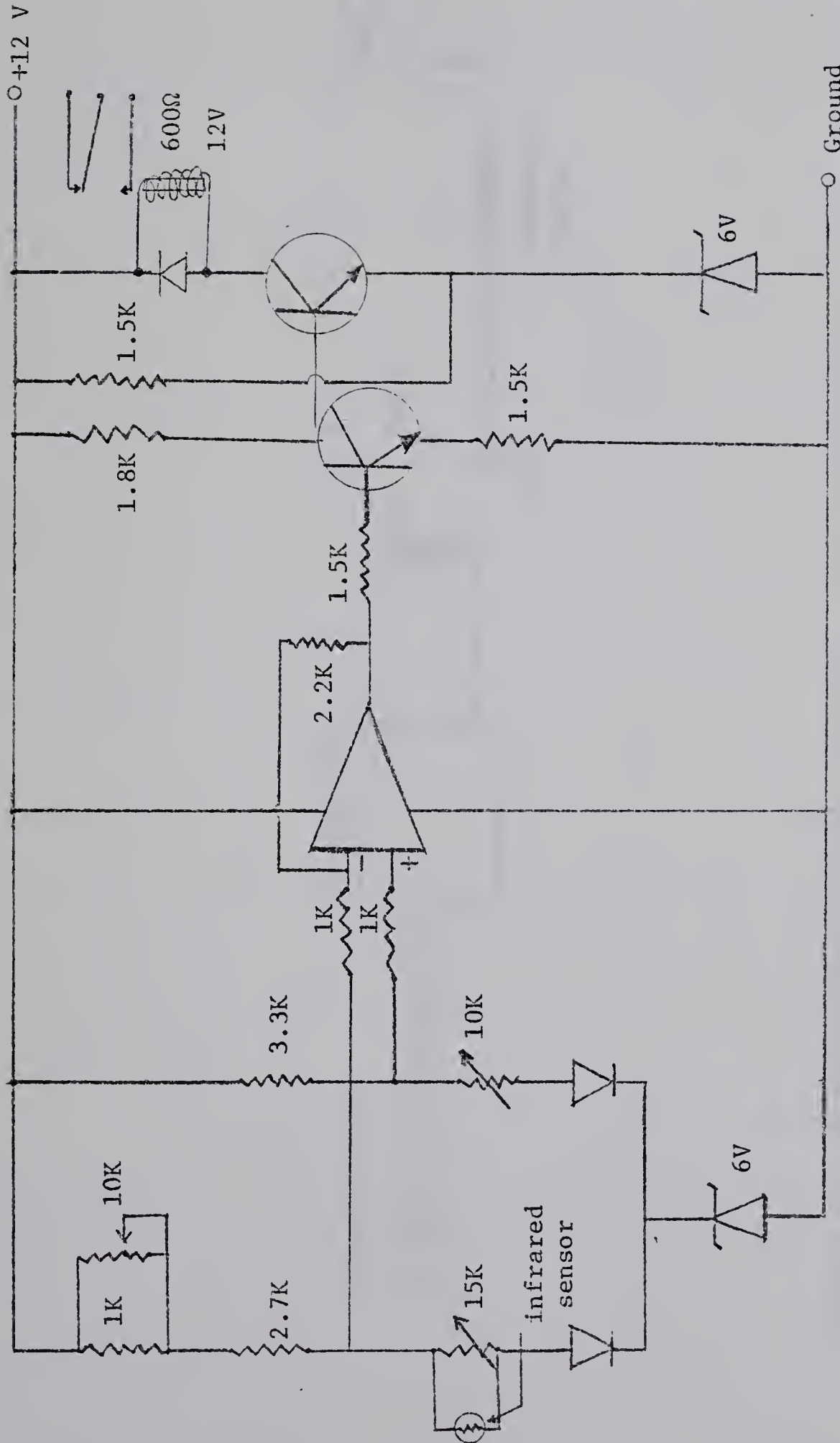


Figure 5: Circuit diagram for the controller, regulating CO₂ concentration in the growth chamber (G of Fig. 4).

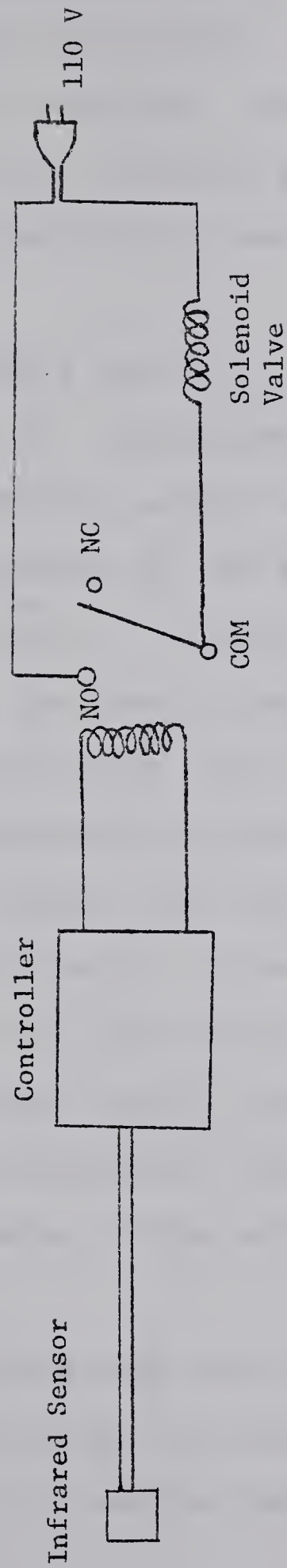


Figure 6: Diagram showing the circuitry for the control of the solenoid valve regulating the CO_2 flow into the growth chamber (G and H in Fig. 4).

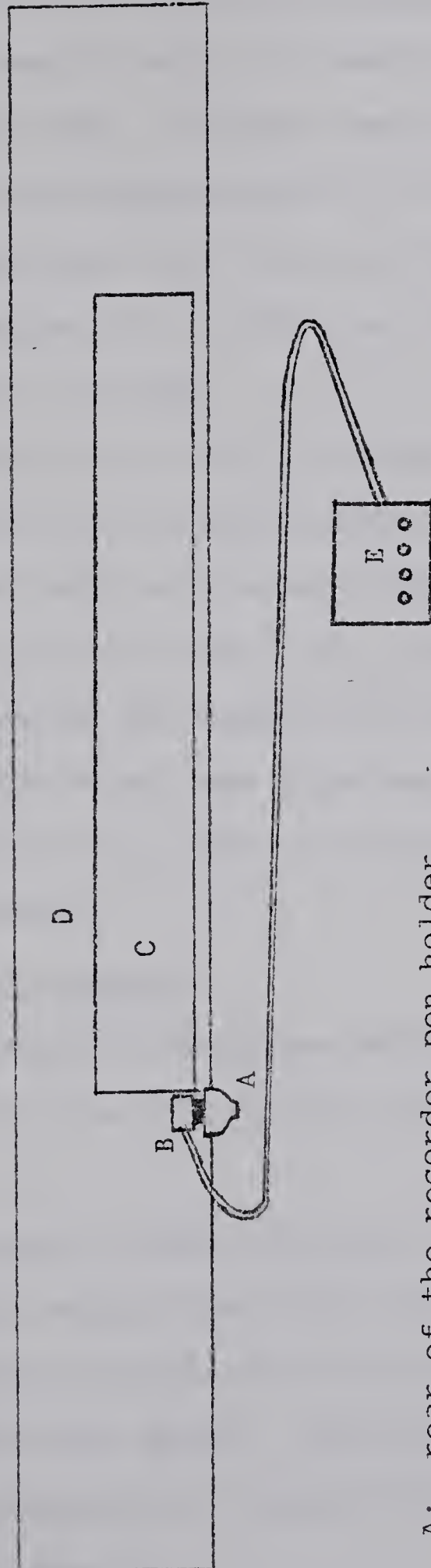
The infrared sensor was mounted on the back of the recorder's pen holder. A strip of white tape was taped on the recorder, one end of which was placed at the front of the infrared sensor when the pen indicated the desired CO_2 conc. (e.g. 1500 ppm, see Fig. 7)

B. CO_2 supply system.

If the CO_2 concentration indicated 1500 ppm or more, the infrared sensor would read the black area so that no activation of the sensor occurred and the relay remained open. If the CO_2 concentration dropped below 1500 ppm, the sensor would read the white tape, activation of the relay would occur causing the solenoid valve (H) to open and CO_2 would flow from the bottled source to the chamber (see Fig. 4).

A needle valve (I) was adjusted to let a very small amount of CO_2 flow into the growth chamber continuously. This kept the CO_2 concentration in the chamber from changing rapidly and reduced the frequency of the opening and closing of the solenoid valve (less wear on the valve).

Bottled commercial grade CO_2 was used for the experiments. The rate of flow of CO_2 out of the bottle was controlled by a needle valve (j). The CO_2 was



A: rear of the recorder pen holder

B: infrared sensor

C: white tape

D: top portion of the recorder chassis

E: controller

Figure 7: Diagram showing the positioning of the controller's infrared sensing unit on the recorder.

directed by two tygon tubes (M) to the circulation fans for maximum mixing within the chamber.

A needle valve (Q) controlled the flow of air sample to the IRGA. Another needle valve (P) controlled the flow of the reference gas (L) to the IRGA for calibration. The third one (R) controlled the flow of zero gas (nitrogen gas)(S). Only one of the three valves was opened at any one time.

Regulation of CO_2 concentration in the growing area continued on a 24-hour basis throughout the 27 days of each experiment with brief stops at day 7, 14, 21 for seedling or plant harvest. The readout of the CO_2 concentration inside the chamber was maintained at 1500 ± 30 ppm throughout all the experiments. Twice weekly recalibration of the IRGA was necessary to maintain this level of control.

XI. Nutrient Solution

Hoagland's solution modified by Johnson, 1957 (Epstein 1972) was used in the first experiments. See Appendix I.

Reagent grade chemicals and distilled water were used for making the stock solutions. Six stock solutions were required with no stock solution being kept more than one month. Approximately 40 liters of nutrient solution were required every two days for the twice daily watering.

Tap water was used in these experiments except for the preparation of stock solutions.

After the first experiment, it was suspected that a high level of NH_4^+ was causing bitter tasting lettuce. Therefore the nutrient solution was modified to include $\text{Ca}(\text{NO}_3)_2$ as the only source for Ca and N and KH_2PO_4 as the source for K and phosphate. The quantity of MgSO_4 was unchanged. Appendix II gives the detailed macro-element components of the modified solution.

XII. Determination of NH_4^+ and NO_3^- by Steam Distillation

In experiment I, due to the bitterness of all the matured lettuce (regardless of treatment) samples were analyzed for NH_4^+ and NO_3^- concentration and compared with samples of commercially grown butterhead lettuce supplied by Safeway (control). For each treatment, as well as the control, samples were taken from two plants, dried in the oven at 60 C overnight. Then 0.5g samples of oven dry material were treated with 100 ml of 2N KCl, shaken for 1 hour and filtered through #54 filter paper. Five ml aliquots were analyzed for NH_4^+ and NO_3^- by steam distillation method (Black, et al (Eds.) 1965).

XIII. Determination of Chlorophyll Content

Twenty 1.25cm diameter samples of lettuce leaf tissue were taken from two plants within each treatment. Each sample was ground with a pestle and mortar in 100 ml of acetone and centrifuged at 3,000 rpm for 20

minutes. The resultant supernatants were measured at wavelength of 663 nm and 645 nm in a Beckman DB - Grating Spectrophotometer for determination of relative amounts of chlorophyll a and b respectively (Tu, Ford and Krass 1968).

XIV. Determination of Mg and Ca in Lettuce Tissue

Young leaves of lettuce from experiments IV and VII as well as commercially grown butterhead lettuce (provided by Scott National Produce Wholesale) were oven dried at 60C and analyzed for calcium and magnesium on an Atomic Absorption Spectrophotometer. Samples of medium from the various treatments were also analyzed for calcium and magnesium content. This was done to determine if the tipburn symptoms were related to Ca deficiency or Mg induced Ca deficiency (Sonneveld and van den Ende 1975).

XV. Statistical Analysis

In each treatment, 3 plants were taken at harvest time for statistical analysis for either Duncan's multiple range test or analysis of variance (Steel and Torrie 1960).

The data were analyzed for significance at the 5% level ($P=0.05$) or at the 1% level ($P=0.01$).

Summary of environmental conditions used in each experiment

Exp. No.	Light Intensity	Photoperiod (hr) per day	Nutrient Solution	Growing Medium	Temp.	CO ₂ conc.	R.H.
I	4000 fc	*** 16 (4 wks) 24 (4 wks) 16/24 (16-2 wks, 24-2 wks) 24/16 (24-2 wks, 16-2 wks)	Hoagland's modified by Johnson (Appendix I)	Peat-vermiculite	20C constant	1500 ppm	85%
II	** 2000 fc 3000 fc	24	Hoagland's modified by Lang (Appendix II)	"	"	"	"
III	** 2000 fc 3000 fc	16	"	"	"	"	"
IV	2400 fc	16	"	Peat & Peat-vermiculite	"	"	"
* V	2400 fc	16	"	Peat-vermiculite	"	"	"
VI	1000 fc	16	"	"	"	"	"
VII	2400 fc	16	"	" with 1/2 of MgSO ₄	"	"	"

* Chamber divided into two light treatments by use of an aluminum foil baffle.
 ** chamber divided into two light intensity treatments by use of one layer of cheese cloth over 1/2 of chamber.
 *** two growth chambers used, one half of plants interchanged after 2nd week.

RESULTS

Experiment I. Influence of photoperiod on plant growth

The fresh weight of lettuce tops was recorded on a weekly basis under four photoperiod treatments, i.e. 24 (continuous), 16, 16/24, (2 weeks in 16 hours of light, then shifted to continuous light for the rest of the experiment) 24/16 (2 weeks in continuous light, then shifted to 16 hours)(Table 1).

At the end of the first week, plants grown under continuous light (24 hours) had more fresh weight than plants grown under 16 hours of lighting per day, but after the second week the difference was not significant. Significantly higher fresh weights were recorded for lettuce plants grown under 16 hour and 24/16 hour regimes of lighting than for plants grown under continuous and 16/24 hour regimes of lighting (Table 1). Significant differences in dry matter content were also recorded for plants grown under the different photoperiod regimes. However, the plants grown under the 16, 24/16 regimes had the least dry matter content (Table I). The quality of lettuce grown under the four treatments is illustrated in Plate 1 (P 62). Under the 24 and 16/24 photoperiod regimes, plants were bleached. The relative chlorophyll content of the plants is shown in Table 2. The chlorophyll content of each cultivar grown under 16 hours of lighting was treated as a control and

Table 1. Fresh weight (g/plant) and dry matter content (%) of lettuce (cv. Buttercrunch) grown under four photoperiod regimes at light intensity of 4000 fc.

	Days from seeding					
	7		14		21	
photo-period	fw(g)	fw(g)	dw(%)	fw(g)	fw(g)	dw(%)
16	0.04	2.06	8.2 ^b	45.33	135.36 ^b	3.7 ^c
24	0.06	1.68	12.8 ^a	25.77	126.54 ^c	6.1 ^a
16/24 [*]	0.04	2.06	8.2 ^b	39.63	123.38 ^c	5.1 ^b
24/16 ^{**}	0.06	1.68	12.8 ^a	41.59	144.42 ^a	3.8 ^c

* two weeks at 16 hr/day followed by two weeks under continuous light;

** two weeks of continuous light followed by two weeks at 16 hr/day.

Within each column, figures not followed by the same letter are significantly different from each other ($p = 0.05$) according to Duncan's multiple-range test.

arbitrarily assigned the value of 100%. The cultivar Buttercrunch had the most chlorophyll content under the 16 hour photoperiod (Table 2). The chlorophyll content of the cultivar Ostinata grown under the 24 and 16/24 regimes of lighting was less than that of plants grown under 16 and 24/16 hours of lighting; plants grown under the 24/16 light treatment had more chlorophyll content than plants grown under the 16 hour photoperiod. (Table 2).

Due to the bitterness of the lettuce grown in this experiment, the leaves were analyzed for NH_4^+ and NO_3^- content and compared with commercially grown butter-head lettuce. It was found that the bitter lettuce leaves contained much more NH_4^+ than the non-bitter commercially grown lettuce (Table 3).

Experiment II. Influence of light intensity on plant growth under continuous light.

The fresh weight of lettuce plants growing under 2000 fc and 3000 fc was recorded on a weekly basis (Table 4). Analysis of variance indicated that treatment interaction varied with cultivars. The fresh weight was not significantly affected by light intensity alone but was significantly affected by light intensity-cultivar interaction. Plants grown under 3000 fc had relatively more dry matter content than those grown under 2000 fc (Table 5). Plants grown under 3000 fc had more leaves than those grown under 2000 fc (Table 5)

Table 2. Relative chlorophyll levels in leaves of two cultivars of lettuce at light intensity of 4000 fc under four photoperiod regimes.

Cultivar	photo-period	Chlorophyll a ⁺		Chlorophyll b ⁺	
		Absorb- ance at 663 nm	Compar- ative %	Absorb- ance at 645 nm	Compar- ative %
Butter-crunch	16	1.0	100	.365	100
"	24	.69	69	.29	79
"	16/24*	.80	80	.33	90
"	24/16**	.875	88	.32	88
Ostinata	16	.50	100	.18	100
"	24	.22	44	.09	50
"	16/24*	.15	30	.07	39
"	24/16**	.60	120	.29	161

+ based on composite samples with no replications

* two weeks at 16 hr/day followed by two weeks under continuous light;

** two weeks of continuous light followed by two weeks at 16 hr/day.

Table 3. Comparison of soluble nitrogen content in relation to bitterness in lettuce.

Cultivar	photoperiod (4000 fc)	culinary quality	NH ₄ ⁺ conc. (ppm)	NO ₃ ⁻ conc. (ppm)
Butter-crunch	24/16**	bitter	8190	30625
"	16	"	10850	19075
"	16/24*	very bitter	14805	11375
"	24	"	14245	12845
commercially grown Butter-head	control	non-bitter	4706	54372

Table 4. Fresh weight (g/plant) of two cultivars of lettuce grown at two light intensities under continuous light.

Cultivar	Light intensity (fc)	days from seeding			
		7 fw(g)	14 fw(g)	21 fw(g)	27 fw(g)
Butter- crunch	2000	.082	1.45	41.45	112.75
"	3000	.135	2.69	49.13	122.34
Ostinata	2000	.175	4.54	55.69	144.30
"	3000	.195	5.46	70.74	173.26

Analysis of variance indicated a highly significant difference between cultivars, significant effect of light intensity-cultivar interaction, but no significant differences between light intensity treatments.

Table 5. Dry matter content and leaf number (> 1 cm) of lettuce plants of two cultivars grown at two light intensities under continuous light.

Cultivar	light intensity (fc)	dry matter (%)	leaf number
Buttercrunch	2000	6.15	34
"	3000	7.27	56
Ostinata	2000	5.25	42
"	3000	6.29	70

* sample size too small for statistical analysis

When relative chlorophyll content of plants grown at different light intensities under continuous light was compared, it was found that chlorophyll content was higher in plants grown at 2000 fc than those grown at 3000 fc (Table 6).

Experiment III. Influence of light intensity on plant growth and development of tipburn under a 16 hour photoperiod.

The fresh weight of plants grown at 2000 fc and 3000 fc under 16 hour photoperiod was recorded on a weekly basis (Table 7). Analysis of variance indicated that there was no significant effect due to either the kind of cultivar or the light intensity (Table 7). Plants grown under light intensity of 3000 fc appeared to have more leaves than those grown under 2000 fc (Table 8).

Several workers, following investigations on a number of environmental factors related to tipburn formation, have suggested that tipburn symptoms are a consequence of rapid growth rates (Tibbitts and Rao 1968; Cox, et al 1976). A comparison was made between the differences in severity of tipburn due to different light intensities at the same photoperiod regime. Plate 2 shows the visible differences in the severity of tipburn. At light intensity of 3000 fc, 50% of the plants started to show tipburn symptoms on day 19 from seeding. (P 62)

Table 6. Comparative chlorophyll levels in lettuce leaves grown at two light intensities under continuous light.

Cultivar	light intensity (fc)	Chlorophyll a ⁺		Chlorophyll b ⁺	
		Absorb- ance at 663 nm	Compar- ative %	Absorb- ance at 645 nm	Compar- ative %
* O	2000	.51	100	.25	100
O	3000	.44	86	.22	88
** B	2000	.88	100	.40	100
B	3000	.56	57	.28	70

* Ostinata

** Buttercrunch

+ based on composite samples with no replications

Table 7. Fresh weight (g/plant) of two cultivars of lettuce grown at two light intensities under 16 hours of lighting.

Cultivar	light intensity (fc)	days from seeding			
		7 fw(g)	14 fw(g)	21 fw(g)	27 fw(g)
B ^{**}	2000	0.04	1.24	22.16	132.30
B	3000	0.04	1.20	29.93	133.90
O [*]	2000	0.06	1.64	22.75	106.15
O	3000	0.07	2.02	27.40	125.45

Analysis of variance indicated there were no significant differences between cultivars or between light intensity treatments.

* Ostinata

** Buttercrunch

Table 8. Dry matter content and leaf number of lettuce of two cultivars grown at two light intensities under 16 hours of lighting.

Cultivar	light intensities (fc)	dry matter content (%) [#]	leaf number [#]
B ^{**}	2000	5.06	32
B	3000	5.12	52
O [*]	2000	4.97	33
O	3000	5.85	45

sample size too small for statistical analysis

* Ostinata

** Buttercrunch

At 2000 fc, 50% didn't show tipburn until day 24.

Comparison of data from Exp. II & III (different photoperiod treatments).

Analysis of variance indicated that there was no significant plant response to photoperiod or light intensity with the cultivar Buttercrunch (Table 9). However, with the cultivar Ostinata there were highly significant differences in plant response between photoperiod treatments, significant difference due to the light intensity-photoperiod interaction, but no significant difference between light intensity treatments (Table 10).

An attempt to compare the ratio of leaf length and leaf width was made (Table 11). From analysis of variance it was found that the ratio of leaf length to leaf width was significantly greater at lower light intensity than at higher light intensity. Also the ratio was significantly higher when plants were grown under 16 hours of lighting than under continuous lighting. There was no significant response to light intensity - photoperiod interaction (Table 11).

Experiment IV. Influence of two kinds of growing media on the development of tipburn and the analysis for Ca and Mg in tipburned and non-tipburned lettuce tissue.

Analysis of variance indicated that there was

Table 9. Comparison of fresh weight (g/plant) of lettuce (cv. Buttercrunch) grown under continuous light and 16 hours of lighting at two light intensity levels.

light intensity (fc)	Photoperiods	
	continuous light fw(g)	16 hours per day fw(g)
3000	122.34	133.90
2000	112.75	132.30

Analysis of variance indicated there were no significant differences between treatments.

Table 10. Comparison of fresh weight (g/plant) of lettuce (cv. Ostinata) grown under continuous light and 16 hours of lighting at two light intensity levels.

light intensity (fc)	Photoperiods	
	continuous light fw(g)	16 hours per day fw(g)
3000	173.26	125.45
2000	144.30	106.15

Analysis of variance indicated there were significant differences between photoperiod treatments, significant light intensity-photoperiod interaction, but no significant differences between light intensity treatments.

Table 11. The ratio of leaf length to leaf width as affected by light intensity and photoperiod (cultivar *Ostinata*).

light intensity (fc)	Photoperiod	
	16 hours/day	continuous light
2000	1.19	0.84
3000	1.04	0.78

Analysis of variance indicated that there were significant differences between photoperiod and light intensity treatments, but no significant effect from photoperiod-light intensity interaction.

no significant difference in fresh weight due to media differences but significant differences due to cultivar and cultivar-medium interaction (Table 12). All plants showed the tipburn symptoms to some degree. Analyses of the Ca and Mg content in the growing media were made and recorded (table 13). There was no significant difference in the Ca content in the two growing media but vermiculite contains more Mg than peat. Analyses for Ca and Mg in lettuce tissue for plants growing in the two media were also made and recorded as well as for commercially grown lettuce (Butterhead type) (Table 14). It was found that the Ca concentration was higher in the lettuce cultivar Ostinata than in cultivar Buttercrunch, but Mg content was not significantly different in the two cultivars.

When compared to the control (commercial butterhead lettuce) the cultivar Ostinata had the same Ca as the non-tipburn butterhead lettuce, but the Mg content was higher. The cultivar Buttercrunch had less Ca content and more Mg content than the commercially grown lettuce.

Experiment V. Comparison of lettuce plants grown under two different light qualities (i.e. with and without incandescent lamps).

Analysis of variance indicated that there was no significant difference in fresh weight of lettuce growing under the two light treatments (Table 15).

Table 12. The fresh weight (g/plant) of two lettuce cultivars grown in two types of growing medium at light intensity of 2400 fc and 16 hours per day of lighting.

Cultivar	Growing Medium	days from seeding			
		7 fw(g)	14 fw(g)	21 fw(g)	27 fw(g)
B **	peat-vermiculite	0.06	2	35.02	117.39
B	peat	0.06	1.95	32.56	133.19
O *	peat-vermiculite	0.16	3.32	46.48	151.61
O	peat	0.14	3.13	47.13	138.83

Analysis of variance indicated that there were significant differences between cultivars, a significant effect from cultivar-medium interaction, but no significant differences between growing media.

* Ostinata

** Buttercrunch

Table 13. The Mg and Ca content of two growing media before planting and after harvest of lettuce plants.

Medium	Treatments	Ca % of dw	Mg % of dw
peat-vermicu- lite	before planting	4.64	7.02
" "	after harvest	4.82	6.48
peat	before planting	4.92	2.78
"	after harvest	4.58	1.28

Table 14. The Mg and Ca content of two tipburned lettuce cultivars and commercially grown non-tipburned butterhead lettuce.

Cultivar	Conditions	Growing Medium	Ca % of dw	Mg % of dw
Butter-crunch	tipburned	peat-vermiculite	0.15	0.22
"	"	peat	0.14	0.22
Ostinata	"	peat-vermiculite	0.22	0.22
"	"	peat	0.26	0.20
Commercial non-tipburned		-	0.21	0.17

Table 15. The fresh weight (g/plant) of lettuce (cv. Ostinata) grown under fluorescent lamps with and without incandescent lamps, at light intensity of 2400 fc and 16 hours of lighting.

Condition	days from seeding			
	7 fw(g)	14 fw(g)	21 fw(g)	27 fw(g)
with incandescent lamps	0.065	1.54	23.25	117.5
without incandescent lamps	0.054	1.33	24.45	109.3

Analysis of variance indicated that there were no significant differences between treatments.

When comparison of severity of tipburn symptoms was made, it was found that all plants showed the tipburn symptoms to some degree (Plate 3, P63).

Experiment VI. Lettuce plant response to low light intensity, 1000 fc, under a photoperiod of 16 hour/day with cultivar Ostinata.

Plants growing at 1000 fc showed a relatively slower growth rate than those grown at higher intensities (Tables 7 and 16). It was found that Ostinata lettuce growing at 1000 fc required another four days in order to reach the same size as plants grown at 2000 fc or 3000 fc (Table 7). The time when 50% started to show the tipburn symptoms was day 29 from seeding.

Experiment VII. Lettuce plants growing under reduced amount of Mg concentration (1/2 of the usual concentration of $MgSO_4$) at 2400 fc and 16 hour/day photoperiod.

Average fresh weight and Ca, Mg content were recorded in Table 17. It was found that even under reduced Mg concentration, all plants showed tipburn symptoms (Plate 4, P63). From the tissue analysis for Mg and Ca, it was found that Mg content was slightly reduced and Ca content was increased somewhat (Table 17) over plants grown in nutrient solution containing usual amount of $MgSO_4$ (regular Hoagland's solution) (Table 14).

Table 16. The fresh weight (g/plant) of lettuce (cv. Ostinata) grown at light intensity of 1000 fc and 16 hours of lighting.

days from seeding		
27	29	31
fw(g)	fw(g)	fw(g)
45.90	80.13	118.20

Table 17. The fresh weight (g/plant) and Ca, Mg content of lettuce grown with reduced MgSO_4 in the nutrient solution.

Cultivar	Fresh Weight (g)	Ca % of dw	Mg % of dw
Ostinata	129.34	0.31	0.19
Buttercrunch	119.41	0.25	0.19



Plate 1. Quality of lettuce cv. Ostinata at harvest growing under four photoperiod regimes (Experiment I).



Plate 2. Degree of tipburn damage on lettuce cv. Buttercrunch growing under 16 hour photoperiod at 2000 fc and 3000 fc (Experiment III).

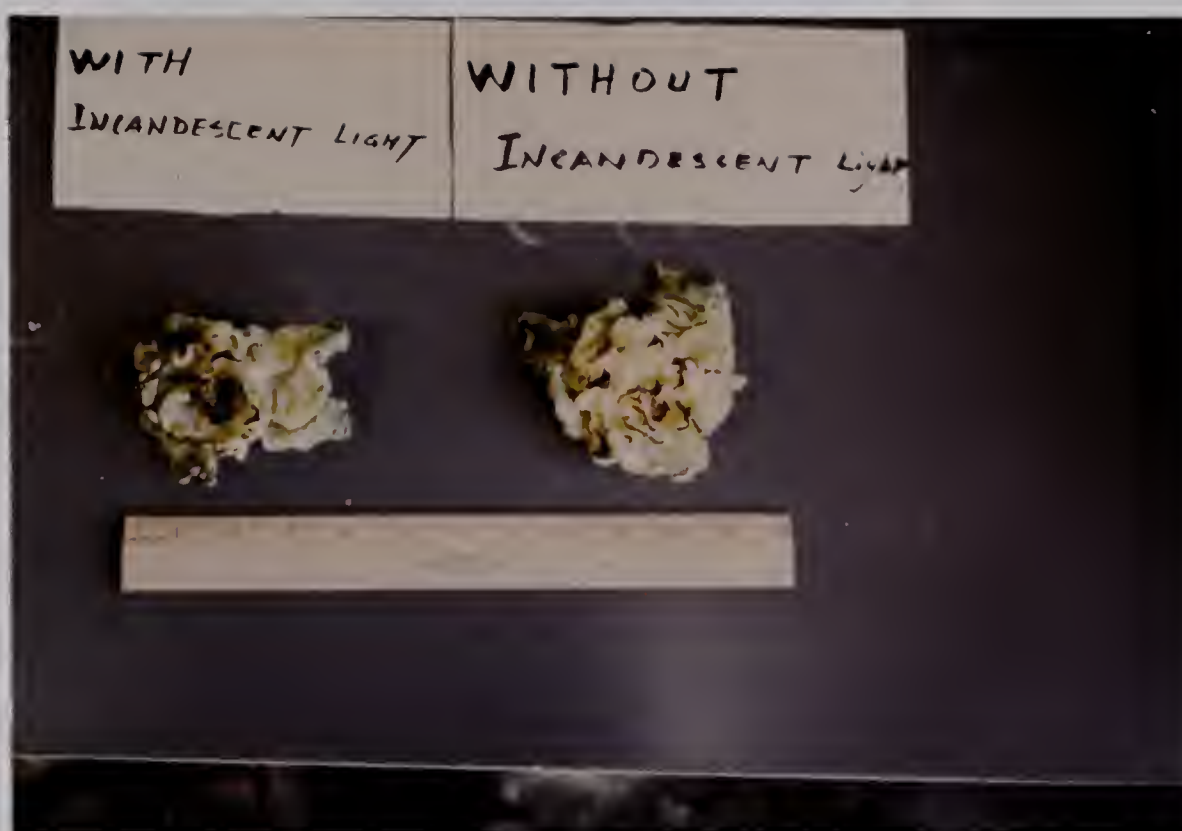


Plate 3. Degree of tipburn on lettuce cv. Ostinata under two light qualities (outer leaves have been removed) (Experiment V).

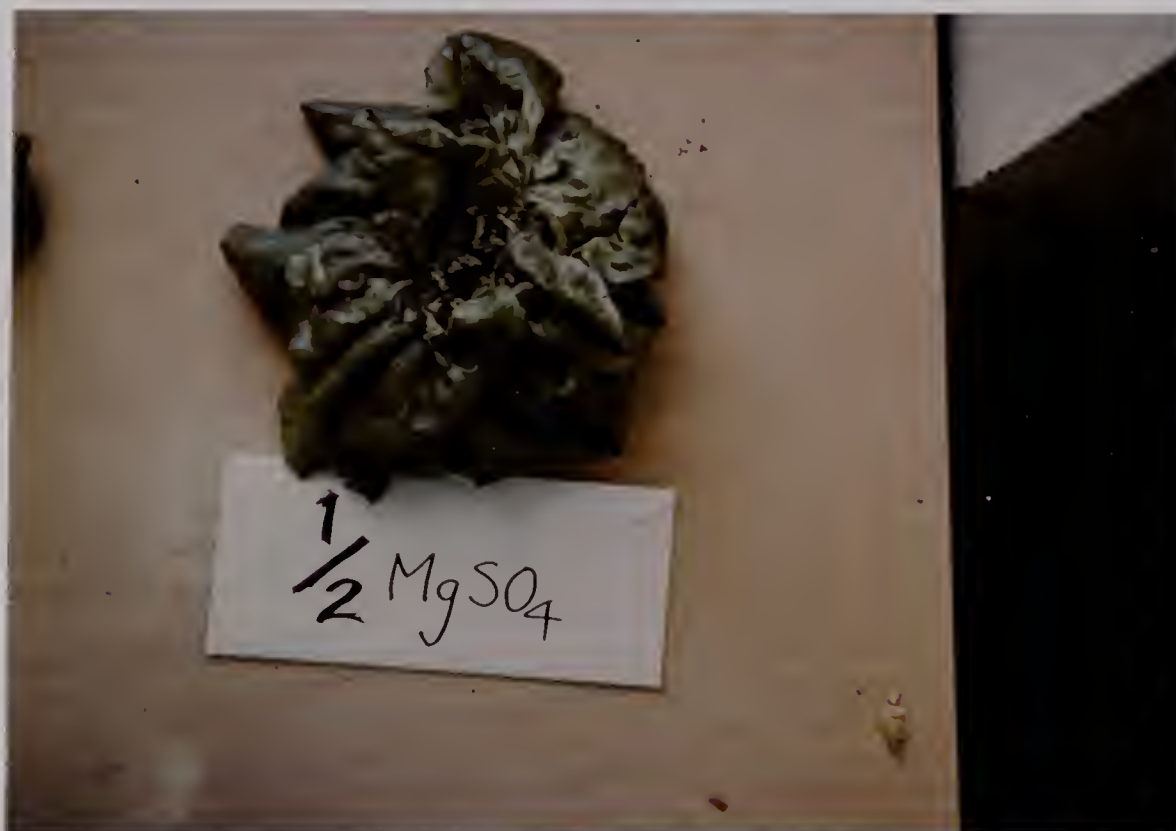


Plate 4. Degree of tipburn on lettuce cv. Ostinata grown in nutrient solution containing one half the normal content of Magnesium sulphate(Experiment VII).

DISCUSSION

Light is a very important factor in plant growth, not only from the standpoint of light intensity, but also photoperiod and light quality. The lettuce grown under continuous light had more fresh weight than that grown under 16 hours of lighting after 1 week of treatment. However, at end of the second week the difference was not significant (Table 1). Furthermore, plants grown under 16 and 24/16 hours of lighting had more fresh weight at harvest (4 weeks) than plants grown under 24 and 16/24 hours of lighting, but the reverse was true for dry matter content (Table 1).

Bate and Canvin (1971) compared the effect of photoperiod on the growth of young aspen trees and found that, provided the photoperiod was longer than that required to maintain active growth, further increases in the length of photoperiod would result in increasing the rate of dry matter accumulation. The net carbon gain was higher in treatments of 24/24, 12/24 than in treatments of 12/12, 24/12.

According to studies at Auburn Rhizotron (root observation chamber at Auburn University, Alabama), plant root extension ceases during the day light hours

and because of low turgor, roots contract and break contact with the soil particles; but after sunset, turgor is restored and extension growth re-established (Leopold and Kriedemann 1975). Therefore, in the current experiment, plants growing under continuous light should have a poorer root distribution than plants growing under 16 hours of lighting and water uptake should be limited.

Extending this line of reasoning further, one can suggest that the plants growing under continuous light have a low fresh weight in relation to dry matter content because these plants produced more dry matter (Bate and Canvin 1971; Table 1), but because of high transpiration (taking place on a 24 hour basis) water uptake does not keep pace with water losses from transpiration, resulting in less water content in plant tissues.

Although plants grown under continuous light contained more dry matter than plants grown under 16 hours of lighting throughout the experiment (Table 1), by week 4 (harvest time) the dry matter content had decreased dramatically for all of the treatments. The rate of decrease for plants under the 24 hour light treatment between week two and week four was 0.5% per day, while the rate of decrease for plants under the 16 hour treatment was only 0.3% per day (Table 1). This

could mean that plants growing under continuous light had a lower photosynthetic rate from week two to week four. This is supported by the fact that plants growing under continuous light throughout the experiment had a reduction in chlorophyll content (Tables 1 and 2). The lower rate of carbon assimilation could also be caused by an imbalance of plant rhythms brought on by the deviation from the 24 hour cycle (Bünning 1952-theory of endogenous daily rhythms).

Because little information could be found on the causes of lettuce bitterness, only an analysis for free NH_4^+ and NO_3^- in the lettuce tissue was performed. This test was run on the supposition that the bitterness principle might be related to nitrogen metabolism. It was found that the NH_4^+ content was higher in bitter lettuce than in non-bitter lettuce, with a good correlation being present between the severity of bitterness and NH_4^+ concentration (Table 3). This accumulation of NH_4^+ suggests a blockage of protein synthesis which could lead to the production of alkaloids or certain "bitter" peptides (Robinson 1968; Arai, et al 1970).

Due to the bitterness of lettuce leaves, a modified solution eliminating ammonium was used (Appendix II) and this effectively eliminated the bitterness in the later experiments. Furthermore,

both light intensity and photoperiod were reduced in these experiments as well. Since high light levels and ammonium sources of nitrogen contribute to the synthesis of alkaloids (Robinson 1968), it is likely that the bitter principle that developed in the lettuce was an alkaloid.

The growth of lettuce is energy dependent, either in terms of light intensity or photoperiod or both (Soffe, et al 1977). Bensink (1971) stated that leaf number is temperature and light energy dependent and at a given temperature, leaf number is proportional to light intensity. Plants grown at 3000 fc appeared to have more leaves than plants grown at 2000 fc (Tables 5 and 8), unfortunately, the samples were not enough for statistical analysis.

Hillman (1956) reported that tomato plants when growing under continuous light at 550 fc as well as at a much reduced light intensity (4 fc) had bleached (chlorotic) leaves. It would appear that this type of chlorosis under continuous light is light intensity dependent. In line with this supposition the author found that at higher light intensity (3000 fc compared to 2000 fc) chlorophyll content was relatively decreased (Table 6). Neither cultivars showed a significant difference in fresh weight when grown at

the two light intensities under 16 hour photoperiod. This is interpreted to mean that there is an energy saturation point for lettuce around 2000 fc. It is suggested by Krizek, et al (1972) that 2000 fc light intensity is optimal for lettuce growth. This is an important economic consideration in terms of capital investment in establishing a commercial growing room.

Analysis of variance indicated that the cultivar Buttercrunch did not show significant response to either light intensity or photoperiod treatments (Table 9). From an economic point of view growing Buttercrunch lettuce at 2000 fc and under 16 hours of lighting should be successful.

The cultivar Ostinata was affected by photoperiod and light intensity-photoperiod interaction (Table 10). One general difference between these two cultivars (Buttercrunch and Ostinata) was the leaf color. Ostinata was light green and Buttercrunch was dark green. Ostinata also had relatively less chlorophyll than Buttercrunch (Table 2). This might be a reason why the growth of the cultivar Ostinata was photoperiod dependent. Furthermore, the cultivar Ostinata showed a more severe loss of chlorophyll under continuous light than did the cultivar Buttercrunch (Table 2).

Consideration of the results obtained, suggest that the optimum photoperiod for the cultivar *Ostinata* may lie between 16 and 24 hours.

Bensink (1971) made a thorough photomorphological study of butterhead lettuce. She showed that the leaf width is positively affected by light energy, either in terms of high light intensities or longer photoperiods. Leaf length on the other hand shows a positive relation with light energy but only at low intensities. At high light intensities, the growth of the midrib is suppressed. A hormone is thought to be the controlling factor. In the current experiment, an attempt was made to compare the ratio of leaf length and leaf width under two different light intensities (i.e. 2000 and 3000 fc) and two photoperiods (i.e. 24 hour and 16 hour of lighting). It was found that plants grown under continuous light at 3000 fc had the smallest ratio. This means that under higher light energy, the growth of the midrib was clearly suppressed and leaf width increased (Table 11). Under the least light energy regime (i.e. 16 hour of lighting at 2000 fc), plants had the greatest leaf length to width ratio, indicating that leaf length had a positive relationship with low light energy. In comparing the plants under the various light treatments, it is evident that the effect of photoperiod on leaf length/width ratio is

predominant at the lower light intensity (Table 11). This is in agreement with the observations of Bensink.

Several papers have been published on the effect of growth rate on lettuce tipburn formation. Some conclusions are: 1) Rapidly growing plants are most susceptible to tipburn when temperature, moisture, light and nutrition are near optimum (Cox, et al 1976; Tibbitts and Rao 1968). 2) High light intensity and temperature cause increased lettuce tipburn (Tibbitts and Bottenberg 1971). The occurrence of tipburn in lettuce has a positive correlation with relative growth rate. Cox, et al (1976) state that the symptoms of tipburn are delayed when light intensity is decreased and the temperature kept constant. In the present work, it was clearly shown that at decreased light intensity, the severity of tipburn was greatly reduced. Furthermore, the initial development of tipburn in plants grown at 2000 fc was 5 days later than that in plants grown at 3000 fc (Plate 2).

Recently, various workers have investigated the relationship between Ca nutrition and tipburn (Ashkar and Ries 1971; Kruger 1966; Thibodeau and Minotti 1969). It has been found that Ca content can be high in the growing medium yet lettuce plants still will develop tipburn symptoms (Table 13). This phenomenon has been reported by other workers using other species of plants. They have found that even when there is an

adequate supply of calcium to the roots, symptoms of calcium deficiency may still occur (Sonneveld and van den Ende 1975; Ashkar and Ries 1971). However, it appears that lettuce tipburn is not associated with Ca deficiency in the usual manner, but rather a temporary localized shortage of soluble Ca during a great need period (Thibodeau and Minotti 1969).

In the present work it was found that Mg content was higher in the medium of peat-vermiculite than in the medium of peat alone (Table 13). However, lettuce still showed the tipburn symptoms when grown in either medium.

Sonneveld and van den Ende (1975) stated that tipburn symptoms will appear even with a slight imbalance in the relationship of the ions (i.e. Na, K, Ca, Mg, P, Cl, N, S). Extensive research was conducted at Naaldwijk research station and it was found that tipburn in lettuce proved to be closely related to the sodium chloride concentration.

The Mg ion could accumulate both in the growing medium (peat-vermiculite) and the lettuce tissue and be a possible cause of tipburn (Tables 13 and 14) (Sonneveld and van den Ende 1975). However, the reduction of MgSO_4 in the nutrient solution resulted in only a slight reduction in Mg content of the plant tissue (Table 17)

and had no effect in reducing tipburn symptoms (Plate 4, p 62)

Furthermore, the tipburn tissue had a large content of Ca (cv. Ostinata) (Tables 14 and 17). Thibodeau and Minotti (1969) stated that lettuce susceptible to tipburn contains a higher ratio of insoluble Ca (Ca oxalate) to soluble Ca in rapidly growing heart leaves than in outer leaves and a possible explanation for this phenomenon is that the environmental factors causing rapid leaf growth not only increase demands for soluble Ca but at the same time accelerate the immobilization of Ca already available, as suggested by Thibodeau and Minotti (1969).

Buttercrunch lettuce tissue had less Ca content than Ostinata (Table 14). Ca deficiency is believed to be the main reason for lettuce tipburn (Ashkar and Ries 1971; Kruger 1966; Thibodeau and Minotti 1969).

Lettuce plants grown at 1000 fc under 16 hours of lighting showed a relatively slower growth rate than those under 2000 fc (Tables 16 and 7) and the visible evidence of tipburn was delayed by 5 days also.

If the tipburn is a consequence of rapid growth rates as suggested by Tibbitts and Rao (1968), Cox, et al (1976), then the high assimilation activity in the leaves associated with rapid growth rate would lead to a demand for certain elements, and deficiency

would occur (Cox, et al 1976). Because the young leaves are most affected, calcium deficiency may be involved rather than other factors associated with rapid growth rate (Cox, et al 1976; Ashkar and Ries 1971; Thibodeau and Minotti 1969).

In addition to calcium deficiency, a deficiency of other ions may cause lettuce tipburn. Lettuce plants grown in a boron deficient nutrient medium will develop tipburn (associated with increase in the activity of auxin-1) (Crisp, et al 1976).

Dr. L.J. LaCroix, Plant Science department, University of Manitoba, stated that wheat growing under supplementary incandescent lamps often shows tipburn-like symptoms, and suggested this may also contribute to lettuce tipburn (personal communication).

From the results of the current experiments, it appears that there was no difference in growth response to light quality treatments (with and without supplementary incandescent lamps). All lettuce plants grown under these two light quality regimes showed tipburn symptoms to some degree (Plate 3, P 63). According to Bate and Canvin (1971), the application of incandescent lamps in growth chambers increases the leaf temperature. Because of the increase in tissue temperature, the tipburn like reaction in wheat seedlings may be induced by high tissue temperature resulting

in "burned" tissues. The tipburn of lettuce is not merely a "burning" of leaf edges.

Tipburn of lettuce is a very complicated problem. The possible causes of tipburn may be:

- 1) Rupturing of laticifers associated with rapid growth rate (Olson, et al 1967; Tibbitts and Read 1976; Cox, et al 1976) and calcium and/or boron deficiency (Ashkar and Ries 1971; Crisp, et al 1976; Kruger 1966; Thibodeau and Minotti 1969).
- 2) Salt accumulation in the growing medium (Sonneveld and van den Ende 1975; Carolus 1975).
- 3) Hormonal influence (Crisp, et al 1976).
- 4) Susceptibility due to genetic control (Crisp, et al 1976).

In lettuce leaves there are meristematic cells located along the two margins of the leaf axis (midrib) called marginal meristems. The activity of these meristems starts about 1/3 down from the tip of the leaf (midrib) and gradually extends along the whole axis (Bensink 1971). From observation, the formation of tipburn appears to originate at the leaf margin just above this marginal meristem.

At high light intensity levels, the assimilation activity in lettuce leaves is very high, resulting in increased assimilates in the laticifers

in young leaves. Furthermore, this increase in assimilates is coupled with rapid growth resulting in increased demands for calcium and/or boron. This in turn can lead to a shortage of soluble Ca resulting in poor cohesiveness between newly forming cells (Ashkar and Ries 1971; Carolus 1975).

The assimilates accumulated within the laticifers increase solute concentration and thus increase osmosis and turgor pressure resulting in laticifer bulging (Tibbitts and Read 1976). Because of a lack of available Ca, plants cells are weak, union of laticifers and parenchyma cells takes place and parenchyma cells fill with latex resulting in leaf necrosis (Olson, et al 1967).

The expression of tipburn susceptibility is neither predictable nor consistent. There is evidence of considerable genotype X environment interaction involved which complicates this problem (Cox, et al 1976). Some suggestions on how to avoid tipburn are outlined below:

- 1) Lowering of the energy input(light

or temperature or both), but this lengthens the growing time required and is not economically efficient

- 2) Foliage application of $\text{Ca}(\text{NO}_3)_2$ as suggested by Kruger (1966), Thibodeau and Minotti (1969). However, when lettuce reaches the heading stage it is inefficient because of the immobilization of calcium and the inaccessibility of the susceptible leaves (Cox, et al 1976).
- 3) Reduction in frequency and amount of nutrient solution applied during later stages of maturity (reduces turgor of laticifers) (Tibbitts and Read 1976).
- 4) Choosing of tipburn resistant cultivars.

Since the plant growth depends upon a continuous supply of raw materials and the influences of environmental factors, such as light, temperature, carbon dioxide, water supply and nutrition, Blackman's (1905) principle should operate. Accordingly, the interaction of these environmental factors should produce a curve showing both exponential and plateau growth.

From the results of these experiments, lettuce

showed a light saturation point around 2000 fc under 1500 ppm CO₂, 20C and modified Hoagland's solution. Based on Blackman's principle, increasing the CO₂ from 1500 ppm to 2000 ppm could result in more dry matter produced. Previous workers have found that CO₂ can be elevated to 2000 ppm without any toxicity symptoms in lettuce plants (Krizek, et al 1972).

Lettuce is a cool season crop. Too high a temperature (above 22C) will cause abnormal growth (Whitaker, et al 1974). Therefore, the temperature of 20C used in these experiments was optimum for rapid growth. Former experiments were conducted under 25C (day)/15C (night) temperature regime but this caused the plants to bolt (Kurelek unpublished thesis). Growing the lettuce at 20C constant temperature effectively eliminates this problem.

Another limiting factor could be the nutrient solution, nutrient solution applied four to six times per day possibly could produce more dry matter as suggested by Krizek, et al (1972).

CONCLUSIONS

From the results of these experiments, a procedure for growing commercial lettuce in controlled environment facilities can be suggested:

Two growth chambers are recommended, one for starting the seedlings and the other for growing the plants on to harvest. This latter chamber should be 4 times larger than the seedling chamber.

Lettuce would be seeded directly to small pots in the seedling chamber and environmental treatments started the day of seeding. Environmental treatments would include 2000 fc light intensity (fluorescent lamps supplemented by incandescent lamps, in input ratio of 80:20 or fluorescent lamps alone), 2000 ppm CO₂, temperature of 20C day/night, nutrient solution applied four times per day and a photoperiod of continuous light for the first ten days. From day 10 to day 14, the photoperiod should be changed to 16 hours per day. The result of the experiment on light quality (Experiment V) indicated no significant advantage in using both fluorescent and incandescent lamps. Therefore, the use of fluorescent lamps only should be successful.

At the end of the second week, the young plants should be transplanted to the harvest chamber to be

grown on to marketable size. In the harvest chamber, the same conditions should be maintained initially as were used in the seedling chamber at time of transfer. At the end of the third week, however, the light intensity should be reduced to 1500 fc, CO₂ levels should be lowered to approximately 500 ppm and the frequency of watering limited to only once per day. The reason for doing this is to curtail the likelihood of having tipburn symptoms develop. As outlined earlier, the development of tipburn is closely associated with rapid growth rate during the heading stage of lettuce.

By following the procedure outlined above, it should be possible to produce butterhead lettuce in 28 to 30 days from seed to harvest.

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APPENDICES

Appendix I. The concentration of each macronutrient elements of Hoagland's nutrient solution, modified by Johnson, 1957 (Experiment I).

Elements	Concentration in
M	
$\text{N}(\text{NH}_4^+)$	2000
$\text{N}(\text{NO}_3^-)$	14000
K	6000
Ca	4000
P	2000
Mg	1000
S	1000

Appendix II. The concentration of each macronutrient elements of Hoagland's nutrient solution as modified by Lang (Experiment II,III,IV,V,VI).

Elements	Concentration in
	M
$\text{N}(\text{NO}_3^-)$	16000
K	6000
Ca	8000
P	6000
Mg	1000
S	1000

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